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Characteristics and Localization of Lipoxygenase Activity in Cucumber (*Cucumis sativus*) Fruit

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Abstract : In order to establish informations important to the measurement of lipoxygenase (LOX) activity, providing conditions most favorable for its action and determining factors that inhibit activity, the influence of extraction buffer, substrate, pH, storage, temperature, NaCl, $CaCl_2$, other cations and antioxidants on LOX activity, and localization of LOX in cucumber tissues were carried out. The most favored substrate for LOX was linolenic acid followed by linoleic and arachidonic acids. LOX activity in both peel and mesocarp tissue extracts was maximum at pH 5.5 and relatively stable at 40℃ and 50℃ temperature. The condition of 0.2 M NaCl with pH 5.0 was observed to provide optimum LOX stability. The enzyme activity was reduced by addition of cations, Mn^{2+}, Cu^{2+} or Al^{3+}, except Ca^{2+} which stimulated activity of LOX. Butylated hydroxy anisole (BHA) and propyl gallate decreased LOX activity with increasing concentration. Cucumber peel had higher activity than other tissues, locule or mesocarp, of cucumber(Received April 29, 1995; accepted October 10, 1995).

Introduction

Physical disruption of cucumbers initiates autolytic changes, catalyzed by endogenous enzymes which cause hydrolytic and oxidative breakdown of lipids to produce volatile compounds with desirable or undesirable flavors. Enzymes involved in generation of volatile compounds are lipoxygenase (LOX) (linoleate: oxygen oxidoreductase, E.C. 1.13.11.12), hydroperoxide lyase (HPL) and isomerase when fresh cucumber fruits are disrupted.[1)] LOX catalyzes the hydroperoxidation of unsaturated fatty acids containing a *cis*, *cis*-1,4-pentadiene system in the presence of oxygen. In cucumber fruit, linoleic and linolenic acids are available to LOX as substrates.[2,3)]

The fatty acid is first attacked by LOX and the product, hydroperoxide, undergoes cleavage and isomerization to form volatilies. LOX is also associated with the development of off-flavors in some plants products, especially in soybeans.[4)] High LOX activity in tomato juice may generate too large a quantity of volatile compounds such as *trans*-2-hexenal and hexanal which are perceived as rancid off-flavors.[5)] Also, nonenzymatic oxidation of unsaturated fatty acids causes off-flavors during storage.[6,7)]

Even though there is production of volatiles, regardless of whether they are originated from macerated tissue or nonenzymatic oxidation, enzyme oxidation is more selective and results in a narrower range of volatiles, which is important in the generation of characteristic cucumber flavor volatiles. Since fresh cucumber flavors are important contributors to flavor in certain cucumber products, it is important to assure their development while inhibiting the formation of adverse flavors.

In order to determine characteristics of LOX which may lead to developing conditions for the generation of good cucumber flavors and for possible control of the development of off-flavor, studies were conducted to investigate the effect of extraction buffer, substrate, pH, storage, temperature, NaCl, $CaCl_2$, other cations and antioxidents on LOX activity. Methemoglobin was used in some of the studies to observe the effects of a nonenzymatic catalyst on linolenic acid oxidation. In addition, localization of LOX in cucumber tissues was determined.

Materials and Methods

Extraction of lipoxygenase

Fresh cucumbers (*Cucumis sativus*) were obtained from the Green Bay Food Company located at Arkins, Arkansas. The cucumbers were washed, sorted for uniformity and freedom from defects, then sealed in polyethylene bags and frozen at −20℃ until used. Fresh-frozen cucumber tissues (10 g of cubed cross-sectional slices) were homogenized with a Tissumizer (Tekmar, Cincinnati, Ohio) for 20 sec in 20 m*l* of citrate-phosphate buffer, pH 6.5. The buffer (a mixture of 0.05 M citric acid and 0.1 M Na_2HPO_4 contained 4 mM dithiothreitol

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(DTT) and 0.2% Triton X-100.[8] The homogenate was filtered through miracloth and centrifuged at 10,000 rpm for 10 min. The supernatant was used as the enzyme source.

Preparation of substrate and assay solution

Unless stated otherwise, linolenic acid was used as the substrate for LOX assays. Linolenic acid (0.15 m*l*) was mixed with 0.15 m*l* of Tween 20, followed by 0.5 m*l* of 1 N NaOH and deionized water. The solution was sonicated until linolenic acid was thoroughly suspended, adjusted to pH 7.0 and the final volume was adjusted to 50 m*l* with deionized water. This stock-substrate mixture was protected against exposure to light and kept refrigerated until used.

To prepare the LOX assay substrate, 1 m*l* of linolenic acid solution was added to 10 m*l* of 0.05 M 2[N-morpholin]ethane sulfonic acid (MES) buffer (pH 5.5) containing 16 mM $CaCl_2$. This solution contained a final concentration of 0.96 μmoles linolenic acid per m*l*.

Lipoxygenase assay

An oxygen electrode (Model 53, Yellow Springs Instrument Company) was used to measure O_2 consumption catalyzed by LOX. The assay solution (2.9 m*l*) was saturated with O_2 at 30℃.[9] The assay was initiated by adding 0.1 m*l* of enzyme extract with a syringe into the stirred substrate. Oxygen consumption was monitored with a recorder, and the rate of activity was calculated from the linear reaction period. Activity of enzyme was expressed as μg O_2 consumed/min/g fresh wt or percent of maximum or control activities.

Effects of extraction buffer, substrate, assay pH and $CaCl_2$

The effects of extraction buffer, assay pH and $CaCl_2$ on LOX activity were determined. Extraction and assay procedures described in previous section were followed.

Linoleic, linolenic, arachidonic and palmitic acids were used as substrates in assay solution. These substrates were prepared by procedures described for preparation of linolenic acid substrate and assay solution in the previous section.

Effects of storage time, temperature and pH on lipoxygenase stability

To establish the storage stability of extracted cucumber LOX, samples were stored at 4℃ for 0, 3, 6, 9, 12 or 16 days and assayed for LOX activity.

In one experiment, LOX extracts (10 m*l*) were incubated for 5, 10, 15 or 20 min at 40, 50 , 55 or 60℃, and in another experiment, the extracts were incubated for 2, 3.5, 5, 10, 15 or 20 min at 65, 70, 75, 80 or 90℃. At the end of each incubation period, the enzyme solutions were immediately cooled in an ice water bath. An enzyme solution not exposed to elevated temperature served as the control.

The effects of pH on LOX stability were determined by incubating LOX extracts (2 m*l*) for 30 min at room temperature in 8 m*l* of 0.2 M acetate buffer at various pH. After incubation, samples were placed in an ice water bath and assayed immediately for activity. Samples held in the respective solutions were also assayed after storage for 1 or 8 days at 4℃.

Effect of NaCl on lipoxygenase stability

To determine the effect of NaCl concentration at pH conditions that may be encountered during cucumber preservation, 2 m*l* of LOX extracts were added to 8 m*l* of 0.04 M citrate-phosphate buffer (pH 3.5 or 5.0) containing 0, 0.063, 0.125, 0.25, 0.5, 1.0, 2.0 or 4.0 M NaCl. Solutions were incubated for 1 hour at room temperature. Samples at pH 5.0 were assayed after storage for 0, 1, 6 or 14 days at 4℃. Samples at pH 3.5 were also assayed after storage for 1, 5 or 11 days at 4℃.

Effects of pH, 2.5% NaCl and $CaCl_2$ on lipoxygenase stability

The effects of pH and 2.5% NaCl on LOX activity was determined. NaCl at 2.5% is typical in many pickle products. Subsequently, the combined effects of pH, NaCl and $CaCl_2$ on LOX activity were determined. Samples were prepared by adding 2 m*l* of LOX extract to 8 m*l* of 0.2 M acetate buffer at pH of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 or 6.5 containing 2.5% NaCl with or without 0.02 M $CaCl_2$. All treatments were incubated for 30 min at room temperature. After incubation, samples were placed in an ice water bath and assayed immediately for LOX activity. Samples were also assayed after storage for 1 or 8 days at 4℃.

Effects of cations, methemoglobin and antioxidants

Cations such as $CuCl_2$, $MgCl_2$, $ZnCl_2$, $MnCl_2$ or $AlCl_3$ (0.2 m*l* of 5, 10, 20 or 40 mM solution) was added to assay solution (2.8 m*l*). Then LOX extract (0.1 m*l*) or methemoglobin (MHG, 0.1 m*l* containing 0.1 mg of MHG) was added and O_2 consumption was monitored. MHG from bovine hemoglobin was commercially obtained from Sigma Chemical Company (St. Louis, Missouri).

Butylated hydroxy anisole (BHA) and propyl gallate at concentrations of 0.25, 0.5, 1.0 or 2.0 mM were also used to observe their effects on linolenic acid oxidation by LOX and MHG. Deionized water was added instead of cation or antioxidant to serve as the control.

Localization of lipoxygenase in cucumber

LOX was extracted from peel, locule and mesocarp tissues of cucumber fruits to evaluate LOX activity in these various tissues. Activity in cross-sectional slices from similar fruits was also measured.

Statistical Analysis

The observations were conducted in triplicate. Significant differences between means were determined by Least Significant Difference option at probabilities less than or equal to 0.05 (SAS User's Guide: Statistics, 1985).

Results

Effects of extraction buffer, substrate and assay pH

LOX activity from cucumber tissues extracted by 0.1 M citrate-phosphate or 0.1 M tris buffer at pH 6.5 or 9.0 was not significantly different (Table 1).

Linolenic acid was observed to provide the greatest LOX activity followed by linoleic and arachidonic acids (Table 2). No activity was observed with palmitic acid, a saturated fatty acid.

LOX activity in both peel and mesocarp tissue extracts was maximum when assayed at pH 5.5 (Fig. 1). At pH 4.5 or less, LOX activity was about 5% of the maximum activity. At pH 6.5, activity was approximately 40 and 60% of the maximum activity for peel and mesocarp tissues, respectively.

Effect of $CaCl_2$ in assay solution

LOX activity was stimulated by addition of 0.1 to 32 mM Ca^{2+} (Fig. 2). Maximum LOX activity was observed with 16 mM Ca^{2+}. In contrast, 64 mM or greater concentrations (data not shown) of Ca^{2+} reduced LOX activity.

Table 1. Effect of citrate-phosphate and tris buffer at pH 6.5 or 9.0 on extraction of lipoxygenase (LOX).

	LOX activity*	
	Extraction pH	
Buffer (0.1 M)	6.5	9.0
Citrate-Phosphate	65.5	74.6
Tris	69.8	71.2

*Activity is expressed as μg O_2 consumed/min/g fresh wt.

Table 2. Effect of fatty acid substrate on lipoxygenase (LOX) activity.

LOX Substrate	Activity*	% of Maximum Activity
Linolenic	85.5 a	100.0
Linoleic	62.3 b	73.0
Arachidonic	17.8 c	20.8
Palmitic	0.0 d	0.0

*Activity is expressed as μg O_2 consumed/min/g fresh wt. Values with same letter are not significantly different by $LSD_{.5}$.

Effects of storage time, temperature and pH on stability

The extracted enzyme (undiluted) was stored for 6 days at 4℃ with little loss (about 3%) in activity (Table 3). After 6 days, activity declined more rapidly. Loss of 51.9% of the original LOX activity was observed after storage for 16 days at 4℃.

LOX activity was relatively stable at 40 and 50℃, but it was unstable at 55℃ and higher (Fig. 3). After incubation for 20 min at 40℃ and 50℃, 94% and 86% of the activity remained in comparison to activity of the 30℃ control. In contrast, 2 min of incubation at 70℃ resulted in about 65% loss in LOX activity, and about 90% was lost after 3.5 min of incubation. Also, 2 to 5 min of incu-

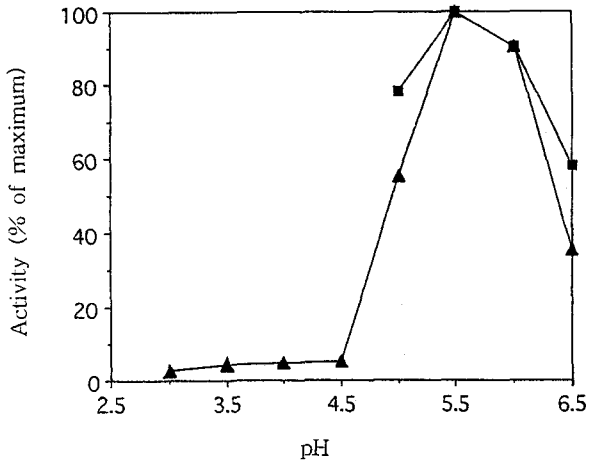

Fig. 1. Effect of assay pH on lopoxygenase activity. Activity is expressed as % of maximum activity at pH 5.5 (100%=27.1 μg O_2 consumed/min/g fresh wt). Peel (▲—▲), Mesocarp (■—■).

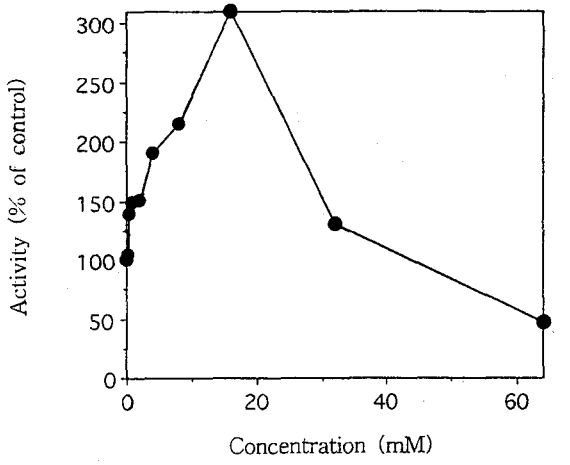

Fig. 2. Effect of $CaCl_2$ concentrations on lipoxygenase activity. Activity is expressed as % of control (9% $CaCl_2$, 100%=65.1 μg O_2 consumed/min/g fresh wt).

Table 3. Storage stability of lipoxygenase (LOX) in cucumber extracts.

Storage Time (Days)	LOX Activity*	% of Total Activity
0	29.5 a	100.0
3	28.6 a	96.9
6	28.5 a	96.6
9	25.1 b	85.1
12	15.5 c	52.5
16	14.2 c	48.1

*Activity is expressed as μg O_2 consumed/min/g fresh wt. Values with same letter are not significantly different by $LSD_{.5}$.

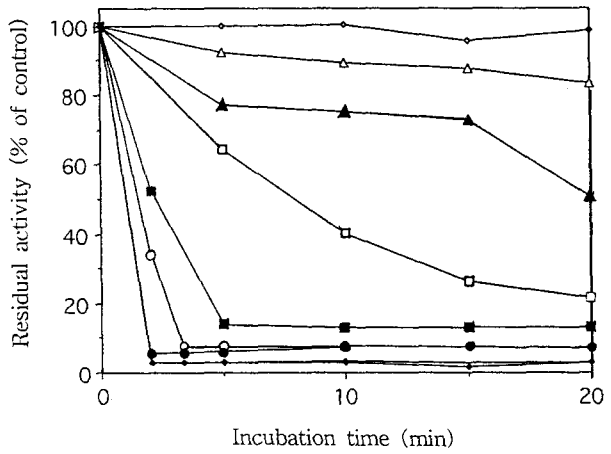

Fig. 3. Effects of incubation times and temperatures on stability of lipoxgenase. Extract were incubated at the respective temperature and times, and then assayed for lipoxgenase activity. Residual activity is expressed as % of activity of nonheated samples, 40℃ (◇—◇), 50℃ (△—△), 55℃ (■—■), 60℃ (□—□), 70℃ (○—○), 75℃ (●—●), 80℃ (◦—◦), 90℃ (◆—◆).

bation at 75, 80 and 90℃ resulted in over 90% loss in activity. Interestingly, about 3% of the original activity appeared to be present even after incubation for 20 min at 90℃. No LOX activity was observed after boiling of the extracts for 10 min which indicated that activity in the 90℃ incubated samples may actually be real.

LOX activity in solutions incubated at pH 3.0 to 6.5 for 30 min declined with decreasing pH (Fig. 4). At pH 3.0, only 63.2% of LOX activity remained relative to maximum activity observed at pH 6.5. After storage for 1 day at 4℃, LOX activity decreased relative to the maximum (30 min incubation at pH 6.5) with decreasing pH below pH 6.0. After 1 day at pH 3.0, LOX activity was only 10.6% of the maximum. After storage for 8 days at pH 3.5 to 5.0, only about 5% of maximum LOX activity remained, but over 30% of maximum activity was observed when held at pH 6.0. No LOX activity was observed when held at pH 3.0 for 8 days.

Effect of NaCl on lipoxygenase stability

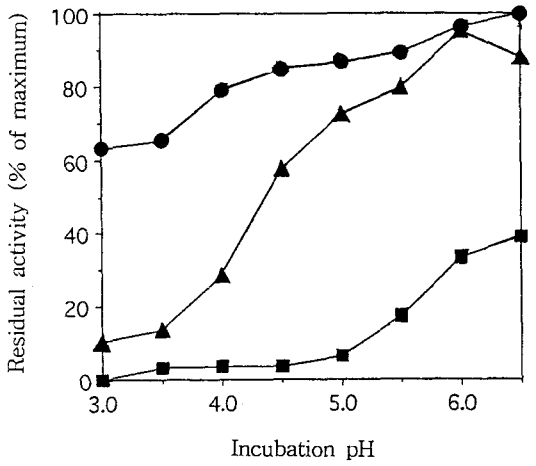

Fig. 4. Effects of incubation pH and times on stability of lipoxygenase. Incubation pH=pH of buffer incubation prior to assay at pH 5.5 Residual activity is expressed as % of maximun activity. (100%=626.9 μg O_2 consumed/min/g fresh wt). Incubation time for 30 min (●—●), 1 day (▲—▲) and 8 days (■—■).

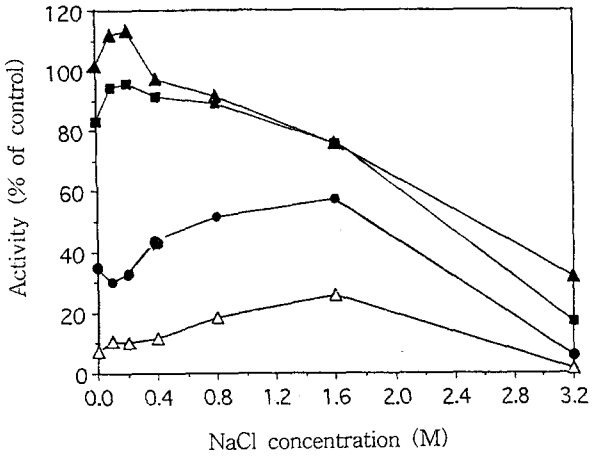

Fig. 5. Effects of NaCl concenytrations and incubation times on stability of lipoxygenase at pH 5.0. Activuty is expressed as % of control. Control=incubation for 1 hour without NaCl (100%=430. 3 μg O_2 consumed/min/g fresh wt). Incubation time for 1 hr (▲—▲), 1 day (■—■), 6 days (●—●) and 14 days (△—△)

The effects of NaCl concentration at pH 5.0 on stability of LOX activity are shown in Fig. 5. Maximum activity was observed when incubated in 0.1 to 0.2 M NaCl at pH 5.0 for 1 hour. LOX activity in extracts incubated for 1 hour at 23℃ was apparently stimulated by NaCl concentrations of 0.2 M or less, but declined with higher NaCl concentrations. Activity decreased during storage at 4℃ in salt solution. After storage for 6 days at 4℃, increasing NaCl to 1.6 M appeared to provide increasing stability, while 3.2 M NaCl for 6 or 14 days at 4℃ was less than 10% of the control activity.

At pH 3.5, the activity after storage for 1 day at 4℃ was stimulated by 0.05 to 0.8 M NaCl (Fig. 6). Maximum activity was achieved with 0.2 M NaCl. Activity decreased

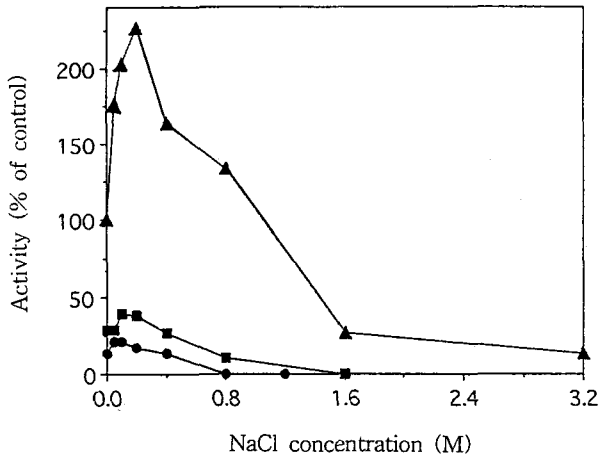

Fig. 6. Effects of Nacl concentrations and incubation times on stability of lipoxygenase at pH 3.5. Activity is expressed as % control. Control=1 day incubation at 4℃ without NaCl (100%=101.1 μg O_2 consumed/min/g fresh wt). Incubation time for 1 day (▲—▲), 5 days (■—■) and 11 days (●—●).

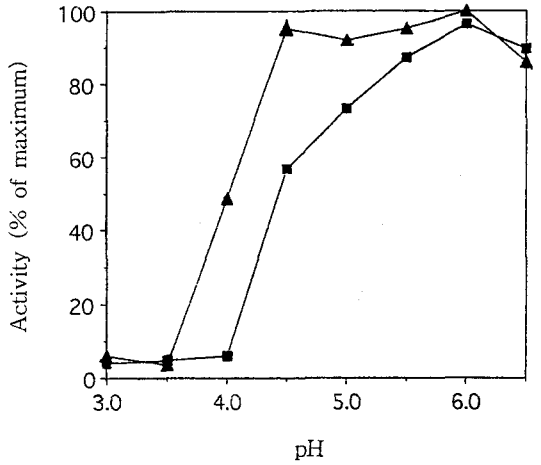

Fig. 8. Effects of incubation pH and times on stability of lipoxygenase at 2.5% NaCl and 0.02 M $CaCl_2$. Activity is expressed as % of maximum activity at pH 6.0 (100%=474.4 μg O_2 consumed/min/g fresh wt). Incubation time for 30 min (▲—▲) and 1 day (■—■).

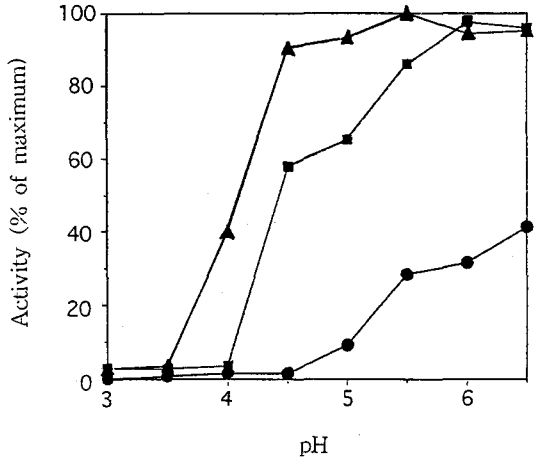

Fig. 7. Effects of incubation pH and times on stability of lipoxygenase at 2.5% NaCl. Activity is expressed as % of maximum activity at pH 5.5 (100%=543.5 μg O_2 consumed/min/g fresh wt). Incubation time for 30 min (▲—▲), 1 day (■—■), 8 days (●—●).

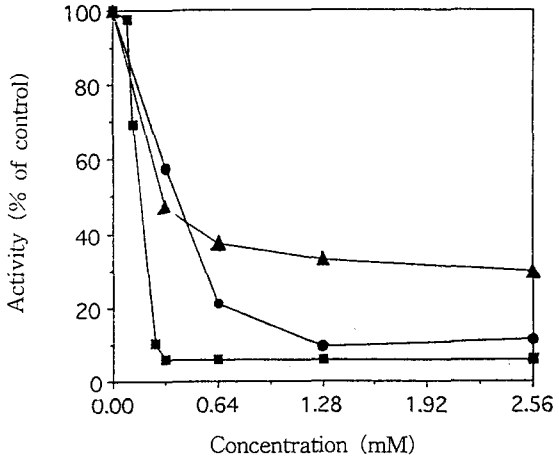

Fig. 9. Effects of Al^{3+}, Mn^{3+} and Cu^{3+} concentrations on lipoxygenase activity. Activity is expressed as % of control (100%=280.1 μg O_2 consumed/min/g fresh wt). Al^{3+} (■—■), Mn^{3+} (▲—▲) and Cu^{3+} (●—●).

with NaCl concentrations greater than 0.2 M. In 1.6 M NaCl, activity was less than 30% of the control activity. After 5 and 11 days storage, LOX activity exhibited a similar pattern of activity in response to exposure to NaCl but at much reduced levels. No activity was detected in extracts with 1.6 M or greater NaCl when stored for 5 days, or when held in 0.8 M or more NaCl for 11 days.

Effects of pH, 2.5% NaCl and $CaCl_2$ on lipoxygenase activity

LOX activity when exposed to 2.5% NaCl declined with decreasing pH from 4.5 to 3.0 (Fig. 7). Between pH 4.5 and 6.5, LOX in 2.5% NaCl remained relatively stable when incubated for 30 min. LOX activity in the extracts stored for 1 day at 4℃ reduced with decreasing pH from 6.0 to 3.5, but it was stable between pH 6.0 and 6.5 relative to the maximum. At pH 3.0, some residual activity (2.5%) was observed in extracts containing 2.5% NaCl and held for 30 min or 1 day.

Storage for 8 days at 4℃ in the pH adjusted solutions reduced LOX activity. Less than 2.2% of LOX activity remained at pH 3.5 to 4.5. No activity in the extracts was observed when stored for 8 days at pH 3.0. Only 41.1% of maximum LOX activity remained after 8 days storage at pH 6.5.

The presence of $CaCl_2$ resulted in a similar pattern to the response of pH on LOX activity when incubated

Table 4. Effect of cations on linolenic oxidation by lipoxygenase (LOX) and Methemoglobin (MHG)

	Activity*			
Cation (2.56 mM)	LOX	% of Control	MHG	% of Control
Control (H_2O)	354.0	100.0	6.5	100.0
Ca^{2+}	357.0	100.8	7.3	112.3
Mg^{2+}	327.0	92.4	7.1	109.2
Zn^{2+}	300.0	84.8	7.4	113.9
Mn^{2+}	177.0	50.0	1.7	26.2
Cu^{2+}	24.0	6.8	12.9	198.5
Al^{3+}	15.0	4.2	9.8	150.8
$Cu^{2+}+Mn^{2+}$	31.0	8.8	12.7	195.4

*Activity is expressed as μg O_2 consumed/min/g fresh wt. for LOX or μg O_2 consumed/min/0.1 mg of MHG.

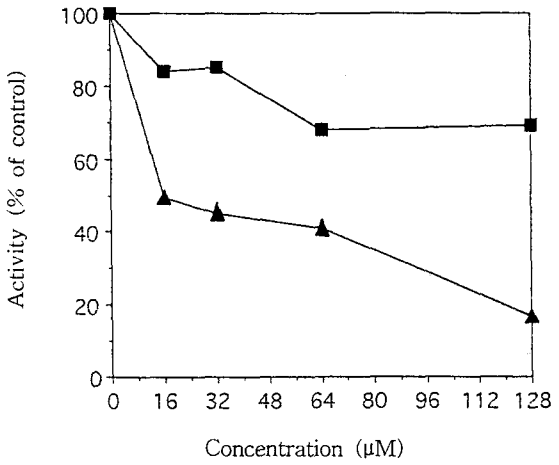

Fig. 10. Effects of butyrate hydroxy anisole (BHA) and propyl gallate concentrations on lipoxygenase activity. Activity is expressed as % of the control (100%=245.1 μg O_2 consumed/min/g fresh wt). Activity is expressed as % of maximum activity at pH 6.0 (100%=543.5 μg O_2 consumed/min/g fresh wt). Incubation pH=pH of buffer incubatuon prior to assay at pH 5.5. Residual activity is expressed as % of maximum activity (199%=626.9 μg O_2 consumed/min/g fresh wt). BHA (■—■) and Propyl gallate (▲—▲).

for 30 min or 1 day (Fig 8). $CaCl_2$ at 20 mM concentration did not appear to influence low pH stability of LOX.

Effects of cations, methemoglobin and antioxidants

LOX activity declined in the presence of Mn^{2+}, Al^{3+} or Cu^{2+} (Fig 9). Activity was inhibited by about 50% with 0.32 mM of Mn^{2+} or Cu^{2+}. With increasing concentration of Mn^{2+} from 0.32 mM to 2.56 mM, activity gradually decreased to 30% of the control. Cu^{2+} caused LOX activity to rapidly decline with 0.32 mM and 0.64 mM concentrations. With 0.64 mM Cu^{2+}, activity was 80% inhibited.

Aluminum ion (Al^{3+}) was particularly inhibitory to LOX activity with maximum inhibition attained with 0.32 mM concentration while maximum inhibition was obtained with 1.28 mM Cu^{2+}. Cu^{2+} and Al^{3+} (2.56 mM) decreased LOX activity by about 95% but tended to stimulate oxidation by MHG more than 150% of the control (Table 4). Ca^{2+} (2.56 mM) had no effect on LOX activity relative to the control. Mg^{2+} and Zn^{2+} (2.56 mM) reduced LOX activity by 7.6 and 15.6%, respectively. Mn^{2+} (2.56 mM) reduced LOX activity by 50% and MHG activity by 73.8%. By combining of Cu^{2+} and Mn^{2+} (2.56 mM), LOX activity was similar to the effects of Cu^{2+} alone.

Table 5. Lipoxygenase (LOX) activity in peel, mesocarp, locule and cross-sectional slices of cucumbers.

Location	LOX activity*
Peel	136.7 a
Mesocarp	19.3 c
Locule	47.3 bc
Cross-section	68.8 b

*Activity is expressed as μg O_2 consumed/min/g fresh wt. Values with same letter are not significantly different by $LSD_{.5}$.

Activity decreased with increasing concentration of BHA and propyl gallate (Fig. 10). BHA was more effective than propyl gallate. At 16 μM concentration, propyl gallate reduced 50.3% of LOX activity, but BHA only reduced LOX activity by 17.1%. Above 64 μM, BHA did not further reduce LOX activity.

Localization of lipoxygenase in cucumber

Cucumber peel tissue contained the highest LOX activity and locule tissue had more activity than mesocarp tissue (Table 5). In cross-sectional slices of cucumber, LOX activity was about half the activity observed in peel tissues.

Discussion

The action of all plant LOX is restricted to substrates which contain a *cis, cis* 1,4-pentadiene structure.[4] In plants, usually linoleic (18 : 2) and linolenic (18 : 3) acid are available to serve as LOX substrates. The fatty acid composition liberated from cucumber lipids are linolenic and linoleic acids.[2,3]

In this experiment, linolenic acid provided the greatest LOX activity followed by linoleic and arachidonic acids. In contrast, Wardale and Lambert reported the most favored substrate for cucumber LOX to be linoleic (100 %), linolenic (77%) and arachidonic (23%) acids.[9] The discrepancy may be explained that in this research, we used sodium linoleate while they used NH_4^+ salts of linoleate.

LOX had maximum activity at pH 5.5. This value is in agreement with pH optimum reported by Wardale

and Lambert.[9] It is known that soybean LOX has isomers with distinctly different pH responses.[10] Optimum activity is achieved at pH 9.0 for LOX-1 and at pH 6.5 for LOX-2. Isoenzymatic forms of LOX also have been identified in other plants.[4] Cucumber LOX exhibited only one optimum pH for activity, however assays were not conducted beyond pH 6.5. Since the pH of cucumber juice is pH 5.5 to 6.0 and physical disruption of cellular constituents is important for LOX reactions (representing conditions of juice extract) then it is doubtful that reactions at alkaline pH would be important to flavor production.

LOX activity was stimulated by Ca^{2+} with 0.1 to 32 mM concentration and maximized with 16 mM of Ca^{2+}. In contrast, Wardale and Lambert reported that calcium (1 mM) did not inhibit LOX activity in crude extracts.[9] Also, Galliard reported that potato LOX was not inhibited by 1 mM Ca^{2+}.[10] It has been reported that cucumber peel tissues contained more LOX activity than flesh tissues.[9] Our results confirmed their report.

LOX extracts held in extraction media were stored for 6 days at 4℃ with minimal loss (about 4%) of activity. With 8 days storage, undiluted LOX extracts had over 85% activity, but LOX activity by about 20% was remained at pH 5.5 as shown in Fig. 4. The discrepancy was caused by 5-fold dilution of LOX extracts.

In this study, LOX stability was highly affected by pH and NaCl. In particular, low pH and high NaCl concentrations reduced LOX stability. However, short time incubation (1 hour) of extracts containing 0.1 to 0.2 M NaCl at pH 5.0 and 0.05 to 0.8 M at pH 3.5 stimulated LOX activity. Also, LOX activity in extracts incubated for 30 min at pH 3.0 to 6.5 declined with decreasing pH. With fixed NaCl concentration (2.5%) at varying pH, LOX activity remained relatively stable for 30 min of incubation at pH 4.5 to 6.5. LOX stability was favored by pH 4.5 and 6.5. LOX stability was favored pH around 6.0 and low concentrations of NaCl. According to the results, LOX activities were most sensitive to pH and NaCl when combined with storage duration.

LOX activity was relatively stable at 40 and 50℃ but it was unstable at 55℃ and higher. Minimal LOX activity was achieved by heating for 10 min at 65℃, 5 min at 70℃ or 2 min at 75, 80 or 90℃.

Pickle products are usually pasteurized at 65 to 70℃ for 10 min to 15 min which should be sufficient to inactivate LOX. Certainly, when the combination of low pH, 2.5% or more NaCl and pasteurization are used in pickle manufacturing, LOX activity would be unlikely to persist. LOX activity was not apparent after boiling the extracts for 10 min.

Cations (except Ca^{2+}) and antioxidants were found to reduce LOX activity. Aluminum ion (Al^{3+}) was particularly inhibitory to LOX activity with maximum inhibition obtained with 0.32 mM concentration. By combining Cu^{2+} and Mn^{2+} (2.56 mM each), we expected to see further reductions in LOX and MHG activity. However, the results were similar to the effects of Cu^{2+} (2.56 mM) alone. Inhibition of LOX activity by Cu^{2+} and Mn^{2+} may be explained in two possible ways. One is the redox reaction between transition metals and non-heme iron of LOX and then transition metals first react with substrates to produce hydroperoxide. Another is the reaction between transition metals and O_2 to reduce oxygen consumption by LOX. Inhibition of LOX by Al^{3+} may be explained that Al^{3+} acts on carboxyl groups of linolenic acid to promote a conformational change in substrate, such as *cis, cis* form to *cis, trans* form which LOX cannot react upon. Pumpkin LOX has also been reported to be strongly inhibited by Cu^{2+} (1 mM).[11]

LOX activity was reduced by BHA and propyl gallate at concentration of 16 μM or more. BHA and propyl gallate act as hydrogen and electron donors and trap the chain propagation peroxy radicals of substrate to reduce oxidation by LOX.

It has been reported that BHA and propyl gallate inhibit LOX activity.[1] In general, all of the conventional antioxidants inhibit plant LOX.[12] Calcium chloride at 20 mM concentration in 2.5% NaCl solution did not increase low pH stability of LOX. This appears to conflict with the earlier statement that 0.1 to 32 mM concentration stimulated LOX activity. However, one study was conducted to observe storage stability of LOX while the other study was conducted to determine the direct influence of Ca^{2+} in assay solution on LOX activity. Calcium ion (Ca^{2+}) in assay solution has been suggested to interact with sodium linolenate to make substrate more accessible to soybean LOX-2.[13,14] But Ca^{2+} inhibits soybean LOX-1.[13] Mildvan[15] reported that Ca^{2+} activates enzyme by forming a substrate bridge of enzyme (E)-substrate (S)-Ca^{2+} type rather than a direct interaction with enzyme of E-Ca^{2+}-S type. Calcium ion (Ca^{2+}) concentration of 64 mM or greater in assay solution reduced LOX activity which might have been due to the formation of precipitates with linolenate. This would reduce the substrate concentration and/or possibly affect the sensitivity of oxygen electrode membrane during assay.

LOX from cucumber was similar in some respects to previously characterized LOX from other plant tissues such as response to pH and sensitivity to various inhibitors. However, the results could not be compared directly as the methods of analysis differed.

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피클용 오이 (*Cucumis sativus*)에 함유된 Lipoxygenase 효소활성의 변화와 효소의 분포 특성

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초록 : Lipoxygenase (LOX) 효소의 최적 활성 조건과 활성 억제등 LOX 활성 측정의 중요한 정보를 확립하기 위하여, 추출 Buffer의 영향, 기질, pH, 저장, 온도, NaCl, $CaCl_2$와 cations 및 antioxidants의 요소들이 LOX 활성에 미치는 영향을 조사하였다. 그리고 오이 tissue내의 LOX의 편재도 시험하였다. LOX에 대한 우수한 기질은 linolenic acid, linoleic acid, arachidonic acid 순서였다. 오이 껍질이나, mesocarp tissue내에 존재하는 LOX의 활성은 pH 5.5가 최적 조건이었으며, 섭씨 40°와 50°에서는 비교적 안정성을 보였다. LOX의 활성은 pH 5.0와 0.2 M NaCl 조건을 같이 주었을때 opitimum 안정성을 보였다. LOX 활성은 Mn^{2+}, Cu^{2+} 또는 Al^{3+}와 같은 양이온에 의해서는 감소되었지만, 오히려 Ca^{2+}은 효소의 활성을 자극시켰다. 한편 butylated hydroxy anisole (BHA)와 propyl gallate의 농도가 증가할수록 LOX 활성은 감소되었다. 오이 껍질에서의 LOX의 활성은 다른 tissue에, locule, mesocarp, 비해 최고치를 보였다.

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